THE OCCURRENCE OF THE LARVAE OF *ONCHOCERCA VOLVULUS* (LEUCKART, 1893), IN THE SKIN OF NATIVES OF THE GOLD COAST

BY

J. F. CORSON

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PLATES XVII AND XVIII

The presence of larvae of *O. volvulus* in the skin has been described by Montpellier and Lacroix (1920, 1921) and by Ouzilleau, Laigret and Lefrou (1921). The former authors stated that the larvae caused itching, resulting in the development of an eruption with papules, vesicles and pustules, which they called filarial itch. The latter authors, on the other hand, considered that the larvae produced an inflammatory reaction in the skin, not especially associated with itching, but giving rise to pseudo-ichthyosis, elephantiasis of the skin of the genital organs and of other parts, leucodermia and atrophy. Brumpt (1920) was not satisfied that the larvae, found in the skin by Montpellier and Lacroix, were those of *O. volvulus*. The observations of Ringenbach and Guyomarc'h (1914), Dubois (1916) and Clapier (1917) are not all in agreement regarding the association or otherwise of elephantiasis with infection with *O. volvulus*.

Larvae of *O. volvulus* were found in the lymph glands by Ouzilleau (1913) and by Fülleborn and Simon (1913). They have since been observed by several others.

In the blood they have very rarely been found. Numerous examinations have been made by Ouzilleau, Rodenwaldt, Rodhain and Van den Branden, Clapier and Montpellier, Lacroix and Boutin, with almost completely negative results. Rodhain and Van den Branden state that Brumpt, in 1904, found them very rarely, that Rodenwaldt found them once among many negative

examinations, and that Ouzilleau found them once only in two thousand examinations. Simon (*loc. cit.*) found that if he squeezed the finger powerfully in making blood smears, larvae of *O. volvulus* were present, and suggested that they were in the lymph that exuded as a result of the squeezing of the tissues.

The following observations were made on prisoners at Seccondee, Gold Coast. An inspection of two hundred and ninety men was made in order to note the presence of subcutaneous tumours and also of abnormal conditions of the skin, especially a dry, glistening, wrinkled appearance, with exaggeration of the normal pattern of lines and intervening areas, the condition termed lichenification. Elephantiasis and pronounced thickening of the skin, 'craw-craw' and signs of scratching were also noted. The results are shown in Table I; in the table, L. = lichenification, E. = elephantiasis, T. = thickening of the skin approaching elephantiasis in degree but not specially localised, C.-C. = craw-craw.

Table I.

Number examined	Subcutaneous tumours present	Abnormal Skin Conditions				
		L.	E.	T.	C-C.	Scratches present
290	16	24	1	3	5	20

Twenty-four cases were selected for investigation; of these thirteen had subcutaneous tumours, and in fifteen the skin showed lichenification. Three of the five cases of craw-craw, the three cases of greatly thickened skin, and the single case of definite elephantiasis of the external genital organs, were also included.

METHOD OF EXAMINATION FOR LARVAE IN THE SKIN

A piece of skin about half a square centimetre in area was excised from the left lower dorsal region in each case and put into a small tube containing normal (0·85 per cent.) salt solution. A bit of the skin was teased and examined soon after excision, the rest being left in the salt solution for a few hours to allow some of the larvae to escape from the skin. The piece of skin was then removed

from the salt solution to 70 per cent. alcohol for subsequent section. The whole of the deposit that formed at the bottom of the tube was put on a slide and examined for the presence of larvae, then fixed by the addition of two or three drops of Ruge's solution (formalin 2 per cent. containing 1 per cent. acetic acid), dried and stained with warm haemalum solution. Drawings and measurements of the larvae were made with the aid of a camera lucida. The chief clinical features and the results of the examination are summarised in Table II, which shows the occurrence in association of subcutaneous tumours, various clinical skin conditions and the larvae of *O. volvulus* in the skin. The same letters as in Table I are used to denote the skin condition.

Table II.

Number of cases	Tumours present	Skin Condition						Larvae of *O. volvulus* in skin
		Normal	L.	L. & E.	L. & C-C.	T.	C-C.	
7	7	...	4	...	2	1	...	7
3	3	3	...	...	...	...	...	3
5	...	...	1	1	...	2	1	5
1	1*	...	1	...	...	...	...	...
2	2†	2	...	...	...	...	...	...
6	...	...	6	...	...	...	...	...
24	13	5	12	1	2	3	1	15

* Aspiration of the tumour failed to show larvae of *O. volvulus*.

† Tumours excised and found not to contain *O. volvulus*.

EXAMINATION OF THE BLOOD

In each case a thick blood smear was taken from the finger both by day and by night. In fourteen of the cases a more thorough examination was also made; six or more fresh blood preparations from the finger and from the back near the place from which the excised piece of skin had been taken were examined. The skin was strongly squeezed also in order to see whether larvae of *O. volvulus* would be readily squeezed out in this way in cases where they were

known to be present in the skin. In three cases about 3 cubic centimetres of blood were withdrawn from a vein and centrifuged, and the deposit examined. Larvae of *O. volvulus* were found in one case only, Case 6, in blood from the skin of the back. The skin in this case was heavily infected, the excised piece yielding about eight hundred larvae to the saline solution in which it was put. This case was one of the three whose blood was centrifuged. In the three cases with greatly thickened skin, and in the case of elephantiasis, thick smears of blood were taken at night from the finger. Embryos of *Filaria bancrofti*, Cobbold (1877), were found in the case of elephantiasis. In the course of the examinations of the blood and of the excised pieces of skin, larvae closely resembling and probably identical with that of *Acanthocheilonema perstans* (Manson), 1891, were seen in twelve cases, and embryos of *F. bancrofti* in three cases.

VITALITY OF LARVAE OF *O. VOLVULUS*

(*a*) *In normal* (0·85 *per cent.*) *salt solution.* The larvae deposited in the tubes of salt solution, when examined a few hours after excision of the pieces of skin, were living and showed active movement. In cover-glass preparations of teased skin, kept in a moist chamber at room temperature (about 25° C.), the larvae were seen to be alive eight hours after removal from the body; on the following day all were motionless. In a case not included in this series, actively moving larvae of *O. volvulus* were found in the skin twenty-two hours after the death of the patient from pulmonary tuberculosis. Larvae obtained by aspiration of a tumour in Case 24 and mounted in salt solution under a cover-glass, ringed with vaseline, showed movements for forty-eight hours.

(*b*) *In blood.* A drop of blood from the skin of the back of Case 6, containing a few larvae of *O. volvulus*, was covered and ringed with vaseline and kept in a moist chamber at room temperature (25° C.). The larvae showed fairly active movement for over five days. Staining subsequently with haemalum confirmed the identity of the larvae. It is interesting to note that Robles (1919) found that the larvae of *Onchocerca caecutiens*, Brumpt, 1919, rapidly died in blood.

IDENTITY OF THE LARVAE IN THE SKIN WITH THE LARVAE OF *O. VOLVULUS*

(*a*) *Morphology*. In the few measurements made of living larvae from the skin, the length varied from 290 to 340μ, the breadth from 6 to 7μ. Stained prepartions showed that they were sheathless, with the cuticle transversely striated for the whole length. The anterior end was free of nuclei for a distance usually of about 10μ; the posterior end was sharply pointed, curved generally at a wide angle, and was free of nuclei for usually the terminal 12 to 15μ. The nuclei were small, mostly oval, longitudinally arranged and closely crowded together, the terminal one being usually distinctly elongated. Of the fixed points of Fülleborn's scheme, the 'nerve ring' and last nucleus were the most easily seen and measured. The G1 cell could not be definitely distinguished in many specimens; in those measured the most frequent position was between 69 and 70 per cent. of the length from the anterior end. Some of these features are shown in Table III, and Tables IV and V give comparative measurements of larvae from tumours.

TABLE III.

Films from the deposit in the tubes containing pieces of skin in salt solution; measurements of 168 specimens, 12 from each of 14 cases.

Length		Relative position of 'nerve ring'		Relative position of last nucleus	
Microns	Number	Percentage	Number	Percentages	Number
235	3	21	3	92·5	2
250	16	22	26	93·5	11
265	46	23	82	94·5	72
280	66	24	52	95·5	78
295	26	25	5	96·5	5

Anterior end free from nuclei		Posterior end free from nuclei	
Microns	Number	Microns	Number
6–8	16	7–11	30
9–12	148	12–16	133
13–16	4	17–21	5

Table IV.

Measurements of 50 larvae from a tumour excised from Case 22.

Length		Relative position of 'nerve ring'		Relative position of last nucleus	
Microns	Number	Percentage	Number	Percentage	Number
225	6	21	1	92·5	1
235	19	22	9	93·5	5
250	17	23	20	94·5	35
265	5	24	15	95·5	8
280	1	25	2	96·5	1
295	1	26	1	...	...
310	...	27–29	...	...	...
325	1	30	1	...	...
...	...	31	...	...	...
...	...	32	1	...	...

Anterior end free from nuclei		Posterior end free from nuclei	
Microns	Number	Microns	Number
6– 7	10	8–10	9
8– 9	32	11–13	30
10–11	8	14–17	11

Table V.

Measurements of 40 larvae from fluid obtained by aspiration of tumours in Cases 2 and 7, 20 from each.

Relative position of 'nerve ring'		Relative position of last nucleus	
Percentage	Number	Percentage	Number
21	1	93·5	10
22	6	94·5	22
23	17	95·5	8
24	13	...	...
25	3	...	...

(*b*) *Relationship to tumours.* In Table II it is seen that larvae were found in the skin in ten of the thirteen cases with subcutaneous tumours. Of the three cases where tumours were present and larvae of *O. volvulus* were not found in the skin, tumours were excised in two and found not to contain *O. volvulus*; in the third case aspiration of the tumour failed to show the presence of larvae. Hence larvae of *O. volvulus* were present in the skin in at least 90 per cent., and possibly in all of the cases with subcutaneous tumours which might be tumours of *O. volvulus*. By excision in Case 22 and by aspiration in Cases 2, 6, 7 and 24, the tumours were shown to contain *O. volvulus*; in these cases larvae were found in the skin. In five cases larvae were present in the skin, but no tumours could be found.

These results confirm the observations of Montpellier and Lacroix and of Ouzilleau, Laigret and Lefrou, that the larvae found in the skin are larvae of *O. volvulus*.

SECTIONS OF SKIN

Larvae of *O. volvulus* were seen in sections of the skin in the papillary and sub-papillary layers at all levels. In many sections they could be clearly seen to be quite apart from the blood vessels. No very marked changes in the skin were observed; there was an excess of cells in the papillary layer and around the capillaries. Otherwise the sections appeared to show little departure from normal.

THE SKIN CONDITIONS AND THEIR RELATIONSHIP TO THE LARVAE

Lichenification was most evident on the back, buttocks and posterior aspect of the thighs; the shoulders and arms were less affected, the chest and abdomen usually still less, and the face, throat and limb flexures hardly at all (Plates XVII and XVIII).

Sweating of the skin was tested in six of the cases; they were set to do hard work in the sun for a few minutes, and in each case sweating of the affected areas was observed.

Itching does not appear to have been severe in most of the cases. One man whose back showed pronounced wrinkling of the skin,

maintained that there was no itching and his skin showed no marks of scratching, yet the excised piece of skin yielded about five hundred larvae to the salt solution in which it was placed.

The number of larvae counted in the smears of the deposit in the tubes of salt solution varied greatly, the greatest number being seven hundred and eighty-five and the smallest two. In Cases 1 and 12 (Plates XVII and XVIII) the numbers were respectively one hundred and seventy-two and five hundred and forty-three. No relationship between these numbers and the degree of skin affection was established. There were six cases with well marked lichenification in whom the excised piece of skin showed no larvae of *O. volvulus*. On the other hand, in three cases with larvae in the skin the latter presented a normal appearance. Larvae were present in the skin in the three cases of 'craw-craw,' and in the four cases with greatly thickened skin, including the case of definite elephantiasis.

In these various conditions there appears to be nothing to indicate a connection between the presence of the larvae of *O. volvulus* in the skin and the appearances observed.

CONCLUSIONS

The following conclusions are drawn:—

1. The larvae in the skin were those of *O. volvulus*.

2. They are present in the skin in all, or nearly all, cases with tumours of *O. volvulus*.

3. No clear causal relationship between the larvae and the conditions of 'craw-craw,' elephantiasis and lichenification was shown in these cases.

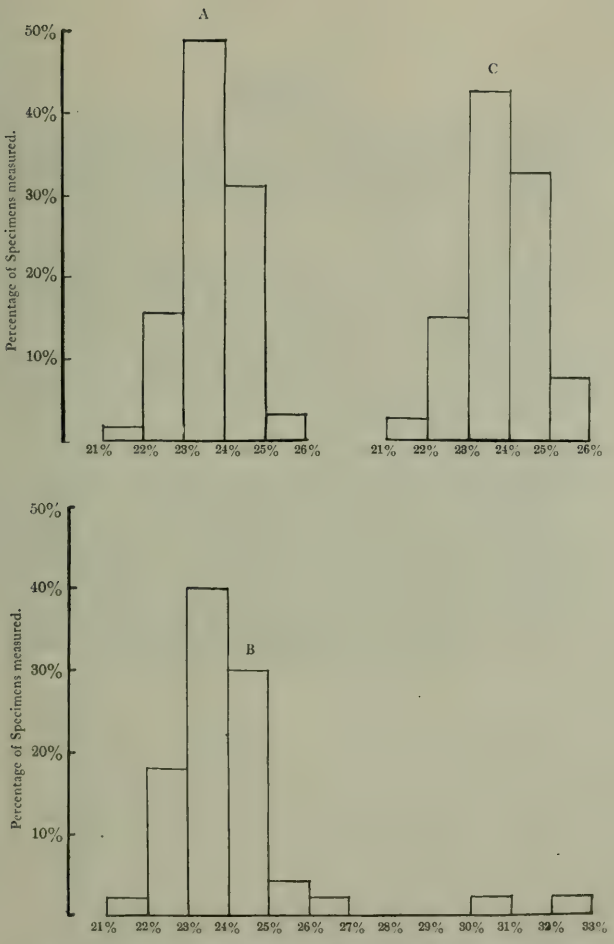

FIG. 1. Comparison of relative position of 'nerve ring' in larvae from (A) skin, (B) excised tumour, and (C) fluid aspirated from tumours; from Tables III, IV and V respectively.

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EXPLANATION OF PLATE XVII

Case 1. Portion of back, showing lichenification of the skin. Three small tumours were present: one behind the great trochanter and two over the lumbar vertebral spines. Larvae of *O. volvulus* were present in the skin.

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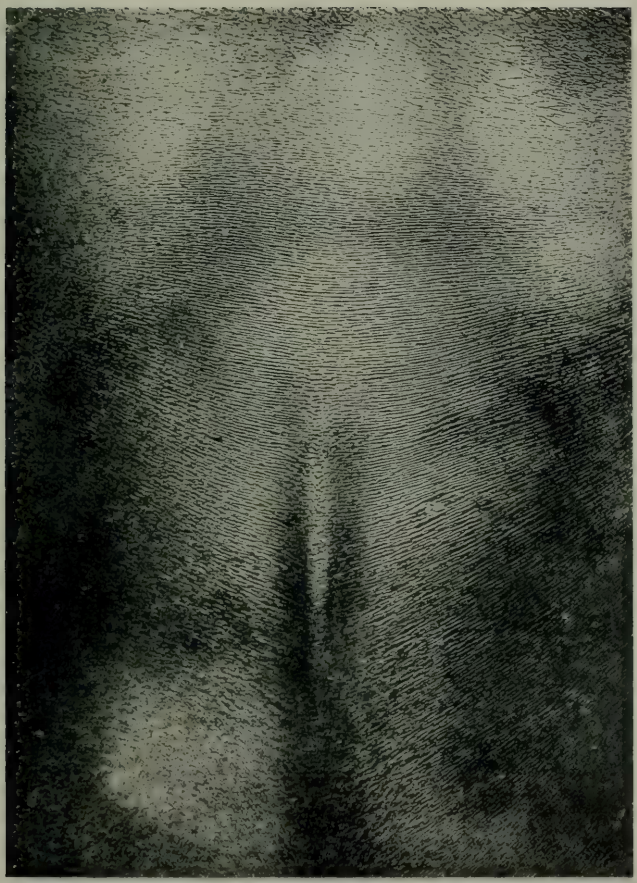

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EXPLANATION OF PLATE XVIII

Case 12. Showing lichenification of the skin. No tumours were found. Larvae of *O. volvulus* were present in the skin. This case had elephantiasis of the external genital organs; the blood contained embryos of *F. bancrofti* and of *A. perstans*.

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